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A NONMANNITOL-FERMENTING TYPE OF S. ENTERITIDIS PRODUCING CLINICAL REACTIONS SIMILAR TO THOSE OF ROCKY MOUNTAIN SPOTTED FEVER VIRUS

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During the summer of 1931 an epizootic occurred among our stock guinea pigs (at the National Institute of Health), killing many of them. The disease in the guinea pig was characterized clinically by fever only. Post-mortem examination showed rather marked congestion of the abdominal viscera, with a spleen enlarged two or three times, darker than normal, and smooth. While febrile, some of these animals were bled from the heart and culture medium was inoculated with the whole blood. In this way there was isolated a strain of S. enteritidis which differed from the strains of S. enteritidis that have been described in that it failed to ferment mannitol. After more than 1 year from the date of isolation, the nonmannitol-fermenting characteristic of this organism persisted. For the purpose of this discussion, this strain of S. enteritidis will be referred to as S. enteritidis 288. A description of this organism follows:

Gram-negative motile rod with peritrichous flagella; grows readily on ordinary culture media; in broth grows readily at room temperature and luxuriantly at 37.0° C.; produces a faint yellow growth on potato in 24 hours; does not liquefy gelatin nor produce indol; hydrogen sulphide is produced in 24 hours; reduces nitrates; in litmus milk it produces acid in 24 hours, less acid in 48 hours, and in 96 hours the milk is alkaline.

Fermentation reactions.—Acid and gas in the monosaccharides, arabinose, rhamnose, xylose, galactose, glucose, mannose, and levulose. In the disaccharide maltose, acid and gas are produced, while lactose, saccharose, and trehalose are unaffected. The trisaccharides, melezitose, and raffinose are unchanged. Acid is produced in the polysaccharides, dextrose, and starch; inulin is unchanged. Of the alcohols, erythretol, adonitol, inositol, and mannitol are unchanged, while acid is produced in glycerol, and acid and gas in dulcitol and sorbitol. The glucocides amygdalin and salicin are unchanged.

Serology.—A study was made of the serological relation between S. enteritidis 288 and two strains of S. enteritidis, two strains of S. aertrycke,

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and one strain each of S. paratyphi, S. schotmuelleri, and B. paratyphosus C. Sera of rabbits which had been inoculated with S. enteritidis 288 agglutinated the enteritidis and not the aertrycke and paratyphoid organisms. S. enteritidis 288 was agglutinated by enteritidis sera and not by aertrycke sera.

Reaction in laboratory animals.-Male guinea pigs were inoculated both with a 24-hour-old broth culture and with a suspension of the organism. The suspensions were prepared from a 24-hour growth on agar slants and were adjusted to a turbidity of 500 parts per million. Following these inoculations the guinea pigs became febrile in from 24 to 48 hours, with maximum temperature ranging from 40° C. to 41.5° C. In addition to the febrile reaction, 25 percent of the animals had definite involvement of the external genitalia characterized by erythema and edema of the scrotum. The post-mortem examination, made in from 2 to 6 days after the onset of symptoms, showed the peritoneum injected and moist; serous fluid in the peritoneal cavity in some and purulent fluid in a few. The spleen varied from slightly to three times enlarged, was darker than normal, smooth, and covered with varying amounts of exudate. The liver was possibly slightly enlarged and darker than normal in some, and in the majority of instances was covered with a filmy exudate. The testicles were normal in size, the vessels of the tunica injected, and in a few instances there was a slight amount of exudate on the testicles.

S. enteritidis 288 could be passed in series in guinea pigs and was carried in this manner for five generations. The transfers were made with whole cardiac blood in doses of 2 or 3 cc, inoculated intraperitoneally. The reactions in the guinea pig following such inoculations differed somewhat from those produced by inoculations of pure cultures of the organisms.

Following inoculations with guinea pig passage S. enteritidis 288, the animals became febrile in from 2 to 6 days. The duration of the febrile period ranged from 4 to 14 days, with a maximum temperature of from 40° C. to 41° C. The scrotal lesion seen following inoculation of the pure culture occasionally occurred in guinea pigs inoculated with the guinea pig passage organism. Post-mortem examinations made on the third or fourth day of fever revealed injection of the peritoneum and a spleen enlarged three to four times, darker than normal, and smooth. No fluid or pus was observed in the peritoneal cavity. The mortality rate of guinea pigs inoculated with the guinea pig passage organism was 20 percent.

S. enteritidis 288 produces in the guinea pig a complete immunity to subsequent inoculations of the same organism.

Three rabbits were inoculated with 0.25 to 0.50 cc of a 24-hour broth culture. Each of the rabbits died within 24 hours.

Twenty-two rabbits were inoculated with guinea pig passage S. enteritidis 288 by means of whole cardiac blood obtained from guinea pigs in the third or fourth day of fever. The injections were made with doses varying from 3 to 8 cc of the blood. Of the 22 rabbits, 2 died too soon to determine any reaction, 14 showed no (or indefinite) reaction, and 6 responded with definite febrile reactions. One of the rabbits reacting with a definite febrile course had a marked lesion of the external genitalia characterized by erythema, cedema and ulceration of the scrotum, and enlargement of the testicles.

CONFUSION WITH ROCKY MOUNTAIN SPOTTED FEVER AND TYPHUS

At the time when the epizootic was occurring among the stock guinea pigs, experiments with the virus of the eastern type of Rocky Mountain spotted fever were in progress. The presence of this S. enteritidis led to confusion. (1) (2).

In the guinea pig, fever is the only clinical manifestation produced by the virus of Rocky Mountain spotted fever isolated from cases occurring in the eastern part of the country, and congestion of the abdominal viscera and enlargement of the spleen are the only gross pathological findings. Rarely erythema and œdema of the scrotum have occurred, but attempts to transmit this involvement to subsequent generations have failed.

In the rabbit the eastern virus of spotted fever produces a febrile reaction, and in some male rabbits a scrotal lesion in addition to the fever. This scrotal lesion is characterized by erythema and œdema of the scrotum which frequently progresses to ulceration and enlargement of the testicles.

From the descriptions given, it is evident that the reactions produced in the guinea pig and rabbit by S. enteritidis 288 simulate those produced by the eastern virus of Rocky Mountain spotted fever. The scrotal lesion produced in the guinea pig by S. enteritidis 288 is, in appearance, more like that produced by the virus of endemic typhus than by the western virus of Rocky Mountain spotted fever.

On account of the confusing clinical reactions in guinea pigs, the immunological relation between this strain of S. *enteritidis* and the viruses of spotted fever and typhus was studied. In testing the immunity of recovered typhus and spotted fever guinea pigs to the S. *enteritidis*, whole cardiac blood of guinea pigs infected with this organism was used. In each instance the S. *enteritidis* was recovered from the blood used in making the inoculations.

Twenty-seven guinea pigs immune either to the western virulent virus or the eastern spotted fever virus, with 49 fresh guinea pigs as controls, were inoculated with this strain of S. *enteritidis*. Seventy and three-tenths percent of the immune animals failed to react (apparently inamune), while but 8.1 percent of the fresh controls failed to react.

Thirty-two guinea pigs immune either to the endemic or epidemic typhus viruses, with 45 fresh guinea pigs as controls, were inoculated with this strain of S. *enteritidis*. Thirty-seven and five-tenths percent of the immune animals failed to react (apparently immune), while but 6.8 per cent of the fresh controls failed to react.

When the guinea pigs immune to S. *enteritidis* were tested for immunity to the viruses of spotted fever and typhus, different results were obtained.

Nineteen guinea pigs immune to S. enteritidis, with 32 fresh guinea pigs as controls, were inoculated with viruses of spotted fever. Ten and five-tenths percent³ of the immune animals failed to react, while none of the fresh controls failed to react.

Seven guinea pigs immune to S. *enteritidis*, with 14 fresh guinea pigs as controls, were inoculated with typhus viruses. None of the animals failed to react.

SUMMARY

A nonmannitol-fermenting strain of S. enteritidis has been isolated from the cardiac blood of guinea pigs during an epizootic.

This strain of *S. enteritidis* produces in the guinea pig and rabbit clinical manifestations similar to those produced by the virus of Rocky Mountain spotted fever isolated from cases occurring in the eastern part of the United States.

An apparently nonspecific immunity occurs between this strain of *S. enteritidis* and the viruses of Rocky Mountain spotted fever and typhus.

This apparent nonspecific immunity has occurred only when guinea pigs immune to the viruses of spotted fever and typhus were inoculated with this strain of *S. enteritidis* and not when immune *S. enteritidis* guinea pigs were inoculated with the viruses of spotted fever and typhus.

This strain of enteritidis, and perhaps other organisms, may cause confusion in isolating a strain of spotted fever or typhus virus.

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³ Not all fresh guinea pigs react to inoculations with viruses of spotted fever and typhus. In a series of 714 fresh guinea pigs, 7.2 percent failed to react to the eastern spotted fever virus, and in a series of 200 fresh guinea pigs 11 percent failed to react to a virus of endemic typhus.

EXPERIMENTAL STUDIES OF NATURAL PURIFICATION IN POLLUTED WATERS

VII. THE SELECTION OF A DILUTION WATER FOR BACTERIOLOGICAL EXAMINATIONS

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In making quantitative determinations of the number of bacteria in a sample which will grow on plates or in tubes, it is usually necessary to dilute a portion of the sample with sterile water to reduce the bacterial content to numbers which can be accurately enumerated. The dilution water may influence bacterial counts thus obtained in at least two ways: It may be bactericidal, and it may contain precipitates which will appear as confusing particles, simulating bacterial colonies on the plates, when the counts are made. Tests for the presence of such interference should be made on the water after it has been sterilized and is ready for use, as the characteristics of a water are frequently changed by sterilization.

In the first (1905) and second (1912) editions of Standard Methods for the Examination of Water and Sewage (American Public Health Association) it is stated that either sterile tap water or distilled water may be used for dilution purposes in bacteriological work. In the third edition and all subsequent editions sterile tap water is recommended and distilled water is not mentioned.

At first thought it would seem that a tap water would be ideal for bacteriological dilution. Upon careful investigation, however, only a few tap waters are found to be entirely suitable. Well, or spring, waters as a rule contain iron and a fairly large amount of mineral salts which are held in solution by carbon dioxide in the water. Precipitates are frequently formed when these waters are exposed to air, and are practically always produced by sterilization with heat. The hydrogen ion concentration of such waters may be changed sufficiently by sterilization to make them bactericidal. Tap waters derived from surface sources are not constant in their composition. They vary not only in their natural mineral salt content but also from the effects of coagulation and chlorination.

In the actual field experience of this laboratory it has frequently been found necessary either to resort to waters other than tap water or to treat the tap water before it was used for bacteriological dilution, because the available tap supply was not suitable after sterilization. For instance, the hydrogen ion concentration of Cincinnati (Ohio) tap water is ordinarily so low after autoclaving, pH 8.6 to 9.5, that it is bactericidal unless it is allowed to stand for at least 48 hours before use to give it time to reach an equilibrium with the carbon dioxide in the laboratory air. In actual practice it is allowed to stand for 96 hours before use. At times when the excess lime treatment is employed to avoid tastes and odors, the water as it comes from the tap is in the pH zone of 9.0 to 9.5, and greater difficulties are encountered. Additional evidence indicating the changes in hydrogen ion concentrations which are produced in waters when they are autoclaved is presented in Table 1. In this table are given the pH values of the various natural and synthetic waters used in this study, as observed before autoclaving, after autoclaving and cooling, and 48 hours after autoclaving. In eight of the eleven waters examined, changes in hydrogen ion concentrations of 0.5 to 1.8 pH were observed.

 TABLE 1.—Hydrogen ion concentration of various synthetic dilution waters and natural waters before and after sterilization by autoclave

	Hydrogen ion concentration expressed in pH			
Water or source of water	Before steriliza- tion	After auto- claving	48 hours after auto- claving	
Distilled	6.7	18.5	7.2	
Bicarbonate	8.0	9.2	8.9	
Phosphate	7.8	7.4	7.4	
Formula U	7.8	7.4	1.4	
Dine Biden S D	7.4	0.0	1.0	
Lake Michigen	8.0	87	82	
Charleston, S. C.	6.4	7.3	7.2	
Lawrence. Kans	7.8	8.2	81	
Lymn. Mass	7.0	8.5	8.2	
Springfield, Ill	7.9	8.6	8.2	

¹ Distilled water changes to pH 7.2 to 7.4 within a short period after sterilization or immediately upon agitation.

Another factor that must be considered in the preparation of bacteriological dilution waters is the quality of the glassware in which the water is sterilized. Collins and Riffenburg (1925) have called attention to the dangers of pollution with materials dissolved from glass. Phosphate-buffered water, pH 7.2, autoclaved in pyrex glass containers, showed an unchanged final hydrogen ion concentration. A portion of the same water after sterilization in a bottle of poor quality glass had a pH of 8.7. Poorly buffered waters undergo still greater changes. All dilution bottles used in this study were tested by filling with distilled water, autoclaving, and then examining the water. The solution of material from glass is most rapid in distilled water. Bottles producing a persistent change in the hydrogen ion concentration of the water were not used.

Similar difficulties have been encountered in selecting a dilution water for use in biochemical oxygen demand determinations. In this instance the criterion for the suitability of the dilution water is even more exacting; for not only must the water be favorable to the continuous

viability of the biological factors concerned, but it must also be free from any substance which would add to the oxygen requirement of the sample or interfere chemically with the quantitative determination of oxygen. Considerable progress has been made toward the development of a dilution water for this purpose. Mohlman, Edwards, and Swope (1928) proposed a dilution water prepared by dissolving 0.5 gram of sodium bicarbonate per liter of distilled water. Later Mohlman (1930) has suggested that the bicarbonate content be reduced to 0.3 gram per liter. Theriault et al. (1931) have reviewed the results presented by these and other workers. In addition they made a comparative study of the oxygen demand results obtained when distilled water, bicarbonate water, and two synthetic waters (phosphate water¹ and Formula C¹ water) prepared by them were used for dilution. As a result of this study, and with a view to the eventual development of a dilution water for general use, they believe that it is desirable to standardize on the readily prepared phosphate dilution water.

As biochemical oxygen demand tests and bacteriological examintions are usually made in the same laboratory, it would be a decided step toward standardization and simplicity of operation if the same dilution water could be used for both procedures. With this object in view, a preliminary study on the selection of a suitable water has been made. In this study the results obtained with Cincinnati tap water, distilled water, bicarbonate water (300 p.p.m.), phosphate water, and Formula C water have been compared to determine the relative suitability of these waters for bacteriological dilution. In each instance the source water was also used as a sixth dilution water. that is, as each sample was received for examination a portion of it was withdrawn, measured into dilution bottles, sterilized, and used as a dilution water for that particular sample. All dilution waters except these source waters were sterilized at least 48 hours before they were used.

In making such a comparison it would be expected that some of the dilution waters might vary in their suitability, depending on the nature and the source of the water sample under examination. In order to test this possibility and to obtain information adapted for more general application, samples for examination, using these dilution

¹ The following stock solutions were used in the preparation of dilution waters:

⁽¹⁾ Phosphate buffer solution (34.0 grams KH_2PO_4 dissolved in 500 ml. of distilled water, adjusted to pH 7.2 with 1 M NaOH solution and made up to one liter with distilled water).

⁽²⁾ Calcium chloride solution, 0.10 M (18.3 grams CaCl₂.4H₂O per liter of distilled water).

⁽³⁾ Magnesium sulphate solution, 0.04 M (9.9 grams MgSO4.7H2O per liter of distilled water).

⁽⁴⁾ Ferric chloride solution, 0.001 M (0.27 grams FeCl_{3.6}H₂O per liter of distilled water).

Phosphate dilution water: The phosphate water was prepared by adding 1.25 ml. of stock phosphate buffer solution per liter of distilled water.

Formula C water: Formula C water was prepared by adding to each liter of phosphate dilution water stock solutions as follows: 0.1 M calcium chloride, 2.5 ml.; 0.04 M magnesium sulphate, 2.5 ml.; and 0.001 M ferric chloride, 0.5 ml.

waters, were obtained from (1) White Clay Creek, Pine Ridge Indian Agency, Pine Ridge, S.Dak.; (2) Lake Michigan, near the mouth of the Chicago River at Chicago, Ill.; (3) Goose Creek, at Charleston, S.C.; (4) Kansas River at Lawrence, Kans. (5) Saugus River watershed at Lynn, Mass.; (6) Sangamon River at Springfield, Ill., and (7) Ohio River at Cincinnati, Ohio. These are fairly representative of waters in the United States, with the exception of the extremely alkaline waters of the far West.

An attempt was made to determine the bacterial count, as observed in the various diluted samples, (1) immediately after the portion of the sample was added to the dilution water, (2) after it had been in the diluting water 15 minutes, and (3) again after a 30-minute exposure. Examinations were not made after longer periods, because in routine procedure the sample would not ordinarily remain in the dilution water for a greater time than 30 minutes before the diluted sample would be mixed with the culture medium.

METHODS

In carrying out these tests the sample was vigorously shaken, and then 1 ml. was withdrawn and added to the first dilution water. The time was noted. The mixture was then shaken vigorously and 1-ml. portions were withdrawn and placed in Petri dishes. Four plates were made of each dilution, and standardized pipettes were used. The plates were poured with agar at once and the time was noted. The diluted sample was then allowed to stand for 15 minutes before it was again vigorously shaken and portions withdrawn for plating. During this 15-minute period the process was repeated with the same sample for each of the dilution waters. The plants for the 15 and 30 minute periods were made in the same manner. The interval during which the portion of the diluted sample was in the Petri dish before agar was added did not exceed one minute. This avoided the adverse effects of sedimentation and evaporation which become a factor if portions of a sample are allowed to stand in plates for 15 minutes or over before the culture medium is added.

Three workers cooperated on each test. One acted as timekeeper, the second made the dilutions and added the 1-ml. portions to the Petri dishes, and the third looked after the agar and poured the plates.

The same agar was used throughout a given test. This agar was melted and cooled to 40° C. before the plating was started and was held at this temperature. Four plates were made of each dilution at each planting. Each count recorded in the tables represents the average of at least four plates from one dilution. The plates were cooled immediately after pouring and mixing, placed in a 37° C. incubator, and incubated for 24 hours before the colonies were counted. Although the exposure of the organisms in the sample to the dilution water, before the addition of agar, was accurately timed, the immediate planting period did not represent a zero time exposure to the diluting water, for some time was required to mix thoroughly the portion of the sample with the water and to add the required portions to the plates. As actually observed, this period of exposure was usually 80 seconds, with an occasional variation of 10 seconds.

A possible source of error that could not be fully controlled was the change that might take place in the number of organisms in the sample during the 9 to 10 minute interval which elapsed while the portions of the sample were being added to the various dilution waters under test. While any change in such a short time would not ordinarily be expected, an attempt was made to balance it by varying the order in which the dilution waters were used in each test. No variations in the initial counts obtained were observed, and it was concluded that the number of organisms in the sample did not change during this period.

RESULTS

The results secured in this comparative study of the bacterial counts obtained on samples from seven different sources, using six different dilution waters for each sample, are presented in Tables 2 to 8.

Dilution waters used	Bacteria per ml. in the diluted sample after indicated intervals					
	Immediate		15 min-	30 min-		
	Actual count	Percent	cent of initial	cent of initial		
Source water	208 195	100 100	96 70	108 67		
Bicarbonate	215	100	60	50		
Phosphate	220	100	89	86		
Formula C	206	100	105	113		
Cincinnati tap	Zł4	100	į ¥3	102		

TABLE 2	2.—Results	with	sample	from	White	Clay	Creek,	Pine	Ridge	Indian	Agency,
			-	Pine	Ridge,	S.D	ik.	• •			

TABLE 3.—Results with sample from Lake Michigan near mouth of Chicago River, Chicago, Ill.

	Bacteria per ml. in the diluted sample after indicated intervals					
Dilution waters used		ediate	15 min-	30 min-		
	Actual count	Percent	cent of initial	cent of initial		
Source water Distilled Bicarbonate Phosphate Formula C Cincinnati tap	124 133 117 137 133 129	100 100 100 100 100 100	135 86 80 86 117 103	137 59 51 84 107 95		

•	Bacteria per ml. in the diluted sample after indicated intervals					
Dilution waters used		Immediate		30 min-		
	Actual count	Percent	cent of initial	cent of initial		
Source water Distilled Bicarbonate Phosphate Formula C Cincinnati tap	152 160 147 146 149 146	100 100 100 100 100 100	97 74 56 97 95 97	98 62 48 96 97 102		

TABLE 4.—Results with sample from Goose Creek at Charleston, S.C.

TABLE 5.—Results with sample from Kansas River at Lawrence, Kans.

	Bacteria per ml. in the diluted sample after indicated intervals					
Dilution waters used		Immediate		30 min-		
	Actual count	Percent	cent of initial	cent of initial		
Source water Distilled Bicarbonate Phosphate Formula C Cincinnati tap	151 130 128 140 143 138	100 100 100 100 100 100	87 95 83 93 108 97	81 82 70 85 84 85		

TABLE 6.—Results with sample from Saugus River watershed at Lynn, Mass.

	Bacteria per ml. in the diluted sample after indicated intervals					
Dilution waters used	Imm	ediate	15 min- utes, per- cent of initial	30 min- utes, per- cent of initial		
	Actual count	Percent				
Source water Distilled Bicarbonate Phosphate Formula C Cincinnati tap	380 369 384 382 392 373	100 100 100 100 100 100	99 74 87 107 101 101	105 47 58 101 104 112		

TABLE 7.—Results with sample from Sangamon River, at Springfield, Ill.

	Bacteria per ml. in the diluted sample after indicated intervals						
Dilution waters used		Immediate		30 min- utes, per-			
	Actual count	Percent	cent of initial	cent of initial			
Source water. Distilled Bicarbonate. Phosphate. Formula C. Cincinnati tap.	213 199 190 201 211 199	100 100 100 100 100 100	105 82 63 114 108 105	106 74 50 109 106 122			

	Bacteria per ml. in the diluted sample after indicated intervals					
Dilution waters used	Imm	ediate	15 min-	30 min-		
	Actual count	Percent	cent of initial	cent of initial		
Source water Distilled Biogroonate Phosphate. Formula C. Cincinnati tap	27 27 24 30 27 27	100 100 100 100 100 100	93 63 67 97 89 93	96 41 54 87 100 96		

TABLE 8.—Results with sample from Ohio River at Cincinnati, Ohio

For convenience in comparing the results, the actual immediate counts have been given in each instance and the subsequent counts obtained at the 15 and 30 minute periods are expressed in percentages of the initial count. This was done in order that possible individual variations in the initial counts obtained with the different diluting waters might not influence the percentages. As a matter of fact, only slight variations were observed in these initial counts, for the average initial counts of the seven samples obtained with each dilution water were as follows: Cincinnati tap water, 175; phosphate water, 179; Formula C water, 180; bicarbonate water, 172; distilled water, 173; and the source waters, 179.

Apparently the relatively short exposure of 80 seconds, of the organisms in the sample to the dilution water before the immediate planting was made, was not sufficient to affect materially the results in any instance. However, when the results for the 15 and 30 minute exposure periods were considered, it is observed that both the bicarbonate and the distilled water gave very low results for both periods with all samples. This general agreement in the results from all samples makes it appear inadvisable to use either distilled or bicarbonate waters for bacteriological dilution at any time. The results obtained from all the samples with the other four dilution waters were about as consistent among themselves as would be expected. The average results for all samples with each dilution water are presented in Table 9.

TABLE 9.—Average results with samples from all sources with each dilution water

	Bacteria per ml. in the diluted sample after indicated intervals					
Dilution waters used	Immediate		15 min-	30 min-		
	Actual count	Percent	cent of initial	cent of initial		
Bource water	179 173 172	100 100 100	102 78 73	105 62 55		
Pacephate. Formula C Cincinnati tap	179 180 175	100 100 100	100 104 99	96 103 106		

Beyond a doubt the bactericidal influence of distilled water was due to the complete absence of mineral salts. Direct microscopic observations on living protozoon cells have shown that these cells are usually ruptured when they are placed in distilled water or in waters with a mineral salt concentration which varies widely from that of the medium in which the organisms are found. It is probable that many bacteria are similarly affected when they are placed in distilled water.

In the case of the bicarbonate water the bactericidal effect might be due to the low hydrogen ion concentration induced in part by heat sterilization, or to the toxicity of the bicarbonate water as prepared. To test this point, bicarbonate water (300 p. p. m.) was prepared in three ways: (1) By sterilizing distilled water and, after cooling and shaking, adding the required amount of sodium bicarbonate with aseptic precautions; (2) by filtration of the bicarbonate solution through a Berkefeld W filter; and (3) by the usual procedure of auto-The hydrogen ion concentrations, expressed in clave sterilization. pH, for the three solutions thus prepared, were 8.1, 8.3, and 9.1, respectively. Using these three bicarbonate waters with sterilized Cincinnati tap water as a control dilution water, samples of Ohio River water were examined. The results obtained are given in Table 10.

TABLE 10.—Results	with sample from	Ohio River at Cincinnati,	Ohio, using bicar-
bona	te dilution waters	sterilized by various means	

	Bacteria per ml. in the diluted sample after indicated intervals					
Dilution waters used	Imm	ediate	15 min-	30 min-		
	Actual count	Percent	cent of initial	cent of initial		
Bicarbonate A ¹ Bicarbonate B ¹ Bicarbonate C ¹ Cincinnati tap	48 48 49 50	100 100 100 100	67 110 98 102	62 100 90 103		

¹ The three bicarbonate waters used were sterilized as follows:

A, by autoclaving in regular manner.
 B, by sterilizing distilled water and preparing solution with aseptic precautions.
 C, by filtering through a Berkefeld W filter.
 Control plates of all three waters were sterile.

These results indicate quite clearly that the bactericidal action of the bicarbonate dilution water was due to the low hydrogen ion concentration and not to the toxicity of the bicarbonate per se.

DISCUSSION

Two prerequisites of synthetic dilution waters for bacteriological use are indicated by the results obtained-the mineral salt content and the hydrogen ion concentration. The results with distilled water

indicate that the presence of some mineral salt is imperative. Of the mineral salts employed in this study, the amount, providing the concentration is within the range found in natural waters, and providing unfavorable hydrogen ion concentrations are not produced, does not appear to affect the results. Hydrogen ion concentrations as low as pH 8.2 were used without reducing bacterial numbers during a 30-minute period. However, at pH 9.0 a very marked decrease in bacterial numbers was observed after 15 minutes' exposure. This indicates that when mixed cultures of bacteria are being considered, many of the bacteria are killed or at least become inactive at a pH between 8.2 and 9.0.

At this point in the consideration of dilution water effects, distinction must be made between the death of the bacterial cell (as evidenced by an inability to grow when transferred from a dilution water to a suitable culture medium), and the ability to grow without lag in a medium diluted with such a water. Butterfield (1929) and Parsons et al. (1929) have shown that certain bacteria in dilute mediums grow best, and without lag, at a pH of about 7.0. Undoubtedly with mixed cultures of bacteria, as the hydrogen ion concentration changes from the zone of pH 7.0, where optimum growth is obtained, to the zone of pH 9.0, where bactericidal effects are observed, varying conditions of growth will be encountered. At the lower pH range the majority of the bacteria will grow well and without lag. In the intermediate zone, growth will occur but with increasing periods of lag. Finally, in the higher pH range many of the bacteria will fail to grow at all or may even be destroyed.

The evidence presented by Theriault et al. (1931, pp. 1099-1100) indicates that when distilled or bicarbonate dilution waters are used for biochemical oxygen demand determinations there is a decided lag in the oxidation rate for at least the first day of incubation. This lag was most marked in the higher dilutions, and the effect persisted longer with distilled water. In the same article (pp. 1112-1113) additional data are presented to show the influence of variations in seeding on biochemical oxygen demand results, being particularly marked during the first day of incubation and occasionally persisting to the end of a test. The data presented at this time, which show that in distilled and in autoclaved bicarbonate dilution water 40 to 50 per cent of the bacterial flora, added in polluted water, are rendered inactive in 30 minutes or less, offer a very probable explanation of the low oxygen demand results obtained when distilled water is used. In the case where unsterilized bicarbonate dilution water is used, the hydrogen ion concentration of the mixture, although not low enough to be bactericidal, is in the zone where a definite lag in the bacterial growth of mixed cultures is induced. Many of the bacteria present in sewage. and probably many protozoa also, find conditions in such

mixtures unsuitable for growth; they pass out of the field of action, and a lag of several hours intervenes before the residual organisms, which are able to survive and to grow, multiply in sufficient numbers to produce normal oxidation. Under such conditions not only is a lag produced by the limited activity during the first day of incubation but the biological flora and fauna acting may also be limited to a lesser number of species and the effect produced by this limitation would persist.

SUMMARY

In this study, in which the results obtained in the bacteriological examination of samples from seven widely separated locations in the United States are compared, using five different dilution waters which have been suggested, the following summary appears to be warranted:

1. Phosphate dilution water and Formula C water give the most consistent results.

2. With a view to the development of a dilution water which can be used for both bacteriological and oxygen demand tests, it seems desirable to standardize on the readily prepared phosphate water for further study, as it forms the basis for the more complete Formula C water.

3. In the bacteriological examination of natural waters the dilution water employed must contain some mineral salts. The amount of mineral salts required in the diluting water, within the range found in natural waters, does not appear to be critical so far as the survival of the bacteria is concerned. If growth of the bacteria, without lag, is desired, it is probable that a degree of mineralization corresponding to that of the natural water would be more favorable.

4. The hydrogen ion concentration of the dilution water also does not appear to require critical adjustment, providing it is not lower than pH 8.2, if survival of the bacteria for a short period only is desired. However if growth of the bacteria without lag is desired, a pH of 7.5 should probably not be exceeded.

5. Distilled water and dilution waters with hydrogen ion concentrations in the zone of pH 9.0 or lower are decidedly bactericidal.

6. In making tests on the suitability of dilution waters, the examinations should be made after the water has been sterilized, for the sterilization process may very greatly alter the characteristics of the water. This is particularly true for tap and bicarbonate waters.

7. Consideration must also be given to the glass container in which the dilution water is sterilized. Material dissolved from glass bottles of poor quality during autoclaving may make marked changes in the reaction and in the mineral salt content of the water.

ACKNOWLEDGMENTS

It is desired to acknowledge the assistance of Elsie Wattie, Orena B. Stewart, Chas. T. Wright, and C. T. Carnahan, of the staff of the Stream Pollution Investigations Laboratory, who aided in carrying out the experimental work reported in this paper.

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COURT DECISION RELATING TO PUBLIC HEALTH

Liability of city for sewage pollution of stream.-(United States Supreme Court; City of Harrisonville v. W. S. Dickey Clay Mfg. Co., 53 S.Ct. 602; decided May 8, 1933.) Since 1923, the city of Harrisonville. Mo., had discharged the effluent from its sewage-disposal plant into a small stream at a point where it flowed through some pasture land owned by the appellee company. The disposal plant consisted of an Imhoff tank and a drainpipe, and it removed about 60 percent of the putrescible organic matter. The cost of the general sewerage system and disposal plant was about \$60,000. A secondary disposal plant, which would have the effect of removing about 30 percent more of the putrescible organic matter, would cost from \$25,000 to \$30,000. The city's population was 2,000, but only about 1,400 of the inhabitants were served by the general sewerage system. In 1928, the clay manufacturing company, a Delaware corporation, brought suit in a Federal court in Missouri against the city, alleging injury to the pasture land through drainage of the effluent and seeking both damages and an injunction. The district court found that the aggregate loss in rental for 5 years was \$500 and that it would cost \$3,500 to restore the creek to the condition existing prior to the nuisance. Damages for \$4,000 were, therefore, awarded, and in addition it was held that the company was entitled to an injunction, but the city was allowed 6 months within which to abate the nuisance

by introducing some method which would prevent the discharge of putrescible sewage into the creek. On appeal by the city to the circuit court of appeals, that court modified the decree by eliminating therefrom the item of \$3,500 damages. The city then carried the case to the United States Supreme Court, not questioning the propriety of the award of \$500 damages but contending that the injunction should have been denied. The Supreme Court took the view that complete monetary redress could be afforded the company "by making denial of an injunction conditional upon prompt pavment, as compensation, of an amount equal to the depreciation in value of the farm on account of the nuisance complained of." The decree was reversed and the cause remanded to the district court for further proceedings to determine the depreciation in value and to enter a decree withholding an injunction if such sum be paid within the time to be fixed by that court. Portions of the Supreme Court's opinion follow:

First. The discharge of the effluent into the creek is a tort; and the nuisance, being continuous or recurrent, is an injury for which an injunction may be granted. Thus, the question here is not one of equitable jurisdiction. The question is whether, upon the facts found, an injunction is the appropriate remedy. For an injunction is not a remedy which issues as of course. Where substantial redress can be afforded by the payment of money and issuance of an injunction would subject the defendant to grossly disproportionate hardship, equitable relief may be denied although the nuisance is indisputable. This is true even if the conflict is between interests which are primarily private. * * Where an important public interest would be prejudiced, the reason for denying the injunction may be compelling. * * * Such, we think, is the situation in the case at bar.

If an injunction is granted, the courses open to the city are (a) to abandon the present sewage disposal plant, erected at a cost of \$60,000, and leave the residents to the primitive methods theretofore employed, if the State authorities should permit; or (b) to erect an auxiliary plant at a cost of \$25,000 or more, if it should be legally and practically possible to raise that sum. That expenditure would be for a desirable purpose, but the city feels unable to make it. On the other hand, the injury to the company is wholly financial. * * * Denial of the injunction would subject the company to a loss in value of the land amounting, on the basis of the trial court's findings, to approximately \$100 per year. That loss can be measured by the reduction in rental or the depreciation in the market value of the farm, assuming the nuisance continues, and can be made good by the payment of money. The compensation payable would obviously be small as compared with the cost of installing an auxiliary plant, for the annual interest on its cost would be many times the annual loss resulting to the company from the nuisance. Complete monetary redress may be given in this suit by making denial of an injunction conditional upon prompt payment, as compensation, of an amount equal to the depreciation in value of the farm on account of the nuisance complained of. We require this payment not on the ground that the nuisance is to be deemed a permanent one, as contended; but because to oblige the company to bring, from time to time, actions at law for its loss in rental would be so onerous as to deny to it adequate relief.

* * * This nuisance has at all times been removable by the device of secondary treatment of the sewage. It may be hereafter abated at any time by the State health authorities requiring such treatment. The city may itself conclude that this should be done in the public interest, financial or otherwise. Being so terminable, pollution of the creek cannot be deemed to be a permanent nuisance as of the date of the installation of the disposal plant in 1923.

DEATHS DURING WEEK ENDED MAY 27, 1933

[From the Weekly Health Index, issued by the Bureau of the Census, Department of Commerce]

	Week ended May 27, 1933	Correspond- ing week, 1932
Data from 85 large cities of the United States: Total deaths. Deaths per 1,000 population, annual basis. Deaths under 1 year of age. Deaths per 1,000 population, annual basis. Deaths under 1 year of age. Deaths per 1,000 population, annual basis, first 21 weeks of year. Deaths per 1,000 population, annual basis, first 21 weeks of year. Data from industrial insurance companies: Policies in force. Number of death claims. Death claims per 1,000 policies in force, annual rate. Death claims per 1,000 policies, first 21 weeks of year, annual rate.	7, 709 10. 8 573 48 11. 8 67, 990, 952 12, 224 9. 4 10. 7	7, 822 11. 2 652 52 12. 3 73, 000, 630 13, 176 9. 4 19. 4

¹81 cities.

173894°-33-2

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

CURRENT WEEKLY STATE REPORTS

These reports are preliminary, and the figures are subject to change when later returns are received by the State health officers

Reports for Weeks Ended June 3, 1933, and June 4, 1932

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended June 3, 1933, and June 4, 1932

	Diph	theria	Infl	uenza	Me	asles	Meningococcus meningitis	
Division and State	Week ended June 3, 1933	Week ended June 4, 1932	Week ended June 3, 1933	Week ended June 4, 1932	Week ended June 3, 1933	Week ended June 4, 1932	Week ended June 3, 1933	Week ended June 4, 1932
New England States: Maine New Hampshire Vermont Massachusetts Rhode Island Connecticut Middle Atlantic States:	1 27 1	1 1 38 4 3	1	4	5 118 62 539 1 289	104 35 358 1,009 32 221	0 0 1 0	0 1 0 2 0 0
New York New Jersey. Pennsylvania East North Central States:	30 20 52	91 25 63	¹ 10 1	¹ 10 7	2, 094 946 1, 257	2, 150 769 1, 629	5 2 0	6 1 14
Ohio Indiana Illinois ‡ Michigan Wisconsin West North Central States:	47 13 34 28 6	31 15 51 18 5	94 25 10 13 26	4 14 58 6 30	613 211 702 640 330	2, 528 125 1, 083 2, 691 1, 570	1 3 29 2 1	5 5 8 3 0
Minnesota. Iowa Missouri North Dakota. South Dakota. Nebraska Kansas South Alantic States:	8 4 15 3 3 3	4 9 23 3 4 8 8	1 2	3 1	248 108 196 268 17 44 261	88 3 61 20 13 7 75	3 1 4 0 1 1 0	1 0 7 1 0 0 1
Delaware Maryland ² ³ ⁴ District of Columbia ³ Virginia West Virginia North Carolina South Carolina ¹ Georgia ³ Florida	6 2 6 4 7 9 1 3	1 5 7 12 5 4	2 2 1 	29 48 249 30 1	14 50 19 214 75 413 252 39	33 20 155 589 214 35 6	0 1 1 1 0 1 0 0 0	0 1 0 2 0 1 0

See footnotes at end of table.

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Cases of	' certain	communicable	diseases	reported	by	telegraph	by	State	health	officers
•	for	we eks ende d Jus	ne 3, 19 3	3, and Ju	nē.	4, 1932 —	Coi	ntinue	d	-

	Diph	theria	Influ	uenza	Me	asles	Menin men	gococcus ingitis
Division and State	Week ended June 3, 1933	Week ended June 4, 1932	Week ended June 3, 1933	Week ended June 4, 1932	Week ended June 3, 1933	Week ended June 4, 1932	Week ended June 3, 1933	Week ended June 4, 1932
East South Central States: Kentucky Tennessee Alabama. Mississippi Vest South Contral States:	4 6 4	6 1 9 4	16 14 14	17 21 32	63 108 56	32 5 8	1 1 1 0	0 3 3 0
Arkansas. Louisiana. Oklahoma ³ . Texas ³ .	3 4 9 30	20 9 27	3 18 3 47	13 6 32 33	240 30 130 412	5 19 337	0 0 2	0 1 0 1
Montani States. Montana ³ Idaho. W yoming ³ Colorado Moria	1	1 10 7		1	28 43 13 16	43 55 126 22	000000000000000000000000000000000000000	001111
New Mexico Arizona Utah 4 Pacific States: Washington	2	2 8	23	6	13 111 48 57	1 2 183	0	000
Oregon ³ California	38	<u>60</u>	17 29	41	47	221 264	0	1
Total	448	619	512	739	12, 570	16, 946	64	72
	Polion	yelitis	Scarle	t fever	Smal	llpox	Typhoi	id fever
Division and State	Week ended June 3, 1933	Week ended June 4, 1932	Week ended June 3, 1933	Week ended June 4, 1932	Week ended June 3, 1933	Week ended June 4, 1932	Week ended June 3, 1933	Week ended June 4, 1932
New England States: Maine New Hampshire Vermont Massachusetts Rhode Island Connecticut	0 0 0 0 0	0 0 1 0 2	18 8 7 253 28 54	7 17 15 366 45 76	0 0 0 0 0 0	0 0 4 0 0 0	1 0 0 7 0 3	2 0 0 7 0 1
Middle Atlantic States: New York. New Jersey Pennsylvania.	2 1 0	3 0 1	478 162 669	984 239 762	0 0 0	0 0 0	4 4 10	9 2 6
East North Central States: Ohio Indiana Illinois ¹ Michigan Wisconsin	0 1 1 1 1	3 0 1 1 0	1, 039 64 375 349 97	328 65 319 503 64	7 0 2 0 0	23 19 7 9 3	24 9 6 4 3	8 9 7 3 0
West North Central States: Minnesota Iowa Missouri North Dakota South Dakota Nebraska Kansas	1 0 0 0 0 0	0 0 0 0 0	81 17 51 3 2 5 31	69 22 29 1 3 15 24	1 14 4 2 0 1 2	5 16 4 1 1 16 5	0 1 2 2 2 3 1	1 0 1 3 0 0
South Atlantic States: Delaware. Maryland ^{2 3 4} District of Columbia ³ Virginia. West Virginia. North Carolina ³ South Carolina ³ Georgia ² Florida.	0 0 0 0 0 0 0 0 0	0 1 0 2 0 2 2 0 0	7 81 10 39 20 34 1 2 0	9 60 14 17 35 7 2 0	0 0 0 1 0 0 0 0	0 0 0 3 1 0 0 0	0 2 0 11 4 18 30 21 2	1 7 0 5 9 20 19 0

See footnotes at end of table.

	Polion	ayelitis	Scarle	t fever	Sma	llpox	Typhoid fever	
Division and State	Week ended June 3, 1933	Week ended June 4, 1932						
East South Central States:								
Kentucky	3	0	27	18	1	1	12	15
Tennessee	i i	i i	23	17	ī	30	11	13
Alahama	l ī	ō	3	4	ī	9	18	5
Mississinni	ō	ŏ	Ā	6	1	5	4	13
West South Central States:	l .			, T	-		i -	
Arkansas	0	0	1	4	0	3	7	5
Louisiana	l ŏ	ŏ	2	10	2	i i	ļ ģ	10
Oklahoma I	Ŏ	Ŏ	6	12	Ō	24	2	7
Teras 2	l ī	i	38	30	12	48	18	3
Mountain States:	-	_						
Montana 3	0	1 0	6	7	0	3	3	4
Idaho	Ŏ	Ŏ	6	l i	2	Ō	Ō	Ō
Wyoming 3	Ō	ĪŌ	16	7	1	l Ó	Ó	Ó
Colorado	Ŏ	9	29	16	Ő	l i	Ō	2
New Mexico	Ō	Ó	5	1 11	Ó	2	3	3
Arizona	Ŏ	Ō	11	6	Ō	1	Ō	Ō
Utah4	Ō	Ó	7	2	0	0	Ó	Ó
Pacific States:	-	-	-	-	_		-	
Washington	0	0	40	26	1	10	0	6
Oregon ³	Ō	Ō	25	10	12	15	4	Ō
California	Ō	3	132	141	28	9	5	8
Total	14	24	4, 368	4, 425	96	279	270	215

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended June 3, 1933, and June 4, 1932-Continued

¹ New York City only.

Typhus faver, week ended June 3, 1933, 23 cases: 2 cases in Illinois, 1 case in Maryland, 2 cases in South Carolina, 5 cases in Georgia, and 13 cases in Texas.
 Rocky Mountain Spotted fever, week ended June 3, 1933, 14 cases: 1 case in Maryland, 1 case in District of Columbia, 2 cases in Montana, 6 cases in Wyoming, and 4 cases in Oregon.
 Week ended Friday.

Figures for 1933 are exclusive of Oklahoma City and Tulsa, and for 1932 are exclusive of Tulsa only.

SUMMARY OF MONTHLY REPORTS FROM STATES

The following summary of cases reported monthly by States is published weekly and covers only those States from which reports are received during the current week:

State	Menin- gococ- cus- menin- gitis	Diph- theria	Influ- enza	Mala- ria	Mea- sles	Pel- lagra	Polio- mye- litis	Scarlet fever	Small- pox	Ty- phoid fever
March 1955										
Colorado	4	28			552		0	173	3	4
Virginia May 1933	9	53	358	10	1, 644	18	2	243	2	29
Connecticut. District of Columbia. Nebraska. New Mexico North Dakota	1 1 2 	10 12 13 25 8	14 1 11 22	7	1, 175 98 747 40 403	2	0 0 0 0	517 51 73 31 33	2 5 1 3	10 1 2 10 2

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Summary of monthly reports from States-Continued

March 1955	May 1955—Continued	May 1955—Continued
Colorado:		
Chicken pox 401	Dysentery:	Sentic sore throat
Mumps 338	Connecticut (becillary) 5	Connecticut 0
Whooping cough 128	New Marico 3	New Mexico
Whooping (s-agn	Common measles	The hard a state of the state o
April 19 33	German measies:	Tracnoma:
Virginia:	Connecticut	Connecticut
Chicken pox	Lethargic encephalitis:	North Dakota
Diarrhea and dysentery 56	North Dakota 1	Trichinosis:
Lethergic encenhalitis A	Milk sickness:	Connecticut
Septia core throat	New Mexico	Undulant fever:
Determine 1	Mumps:	Connecticut 1
	Connecticut 364	Nebreska 2
Tracnoma 1	Nabracka 109	Now Maria
Typhus fever 1	Now Mariao 77	Vincent's anging.
Undulant fever	New Mexico	Vincent s angina:
Whooping cough 158	North Dakota 2	New Mexico 1
Man 1099	Ophthalmia neonatorum:	Vincent's infection:
May 1000	Connecticut 1	North Dakota
Chicken pox:	Paratyphoid fever:	Whooping cough:
Connecticut	Connecticut 1	Connecticut 264
District of Columbia 100	Puerperal septicemia:	District of Columbia 34
Nebraska 174	New Mexico 3	Nebraska 112
New Marico 40	Rabies in enimals.	New Movico 102
North Dekote 54	Connectiont 12	North Dakata
TAOLET TATORS	Connecticut	

WEEKLY REPORTS FROM CITIES

	C	ity re	ports fo	or wee	k ende	d Ma	y 27,	1933			
State and situ	Diph-	Inf	Influenza		Pneu-	Scar- let	Small-	Tuber-	Ty- phoid	Whoop- ing	Deaths,
State and city	cases	Cases	Deaths	cases	deaths	fever cases	cases	deaths	fever cases	cough cases	causes
Maine: Portland	o		0	2	2	7	o	3	0	9	26
New Hampshire: Concord Nashua	0 0		0 0	0 0	2 0	3 0	0	0 0	0 0	0 1	1 2
Barre Burlington Massachusetts:	0 1		0 0	0 0	0	0 0	0 0	0	0 0	6 0	1 7
Boston	3 0 0 2		1 0 0 0	297 0 2 52	22 1 1 3	83 5 10 21	0 0 0 0	8 2 1 4	1 0 0 0	24 4 16 1	214 26 32 56
Rhode Island: Pawtucket Providence Connecticut:	0 1		0	0 2	0	2 16	0	0 2	00	0 23	14 62
Bridgeport Hartford New Haven	0 0		0 0	33 1	1 2	12 3	0 0	4 2	0 0	1 15	32 40
New York: Buffalo New York Rochester Syracuse	5 59 1 0	9	0 9 0 0	85 1, 317 2 3	14 139 4 4	53 221 26 14	0 0 0 0	- 5 91 0 0	0 1 0 0	33 142 11 7	140 1, 403 69 47
New Jersey: Camden Newark Trenton Popperiyania:	1 0 0	i	0 0 0	17 154 36	3 7 0	11 11 14	0 0 0	0 11 3	0 1 0	0 43 2	35 121 21
Philadelphia Pittsburgh Reading	6 2 0	2	2 0 0	509 7 17	20 10 2	85 80 7	0 0 0	23 4 0	1 0 0	3 48 3	429 135 24
Ohio: Cincinnati Cleveland Columbus Toledo	2 8 0 1	2 46 2 1	2 0 2 0	10 4 4 211	10 6 4 4	30 138 29 129	0 0 0 0	9 11 4 6	0 0 0 0	13 30 1 6	133 140 90 77
Fort Wayne Indianapolis South Bend Terre Haute	8 0 0 0		0 1 0 0	0 120 1 48	1 10 1 0	8 15 1 6	0 0 0	0 3 1 1	3 0 0 0	1 12 4 8	26 9 21

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City reports for week ended May 27, 1933—Continued

		· · · · · ·			· · · · · ·						
State and city	Diph- theria	Inf	uenza	Mea-	Pneu- monia	Scar- let	Small-	Tuber-	Ty- phoid	Whoop- ing	Deaths, all
	Cases	Cases	Deaths	cases	deaths	Cases	Cases	deaths	Cases	Cases	causes
This size										, !	
Chicago	2	[2	434	42	273	0	48	0	23	690
Cicero	ō		Ō	18	Ō	4	ŏ	Õ	Ŏ	Ō	3
Springfield	1		• 0	0	0	10	0	0	1	0	15
Michigan:			.		10	194					000
Elimt	13			310	10	134	Ň	24		7	200
Grand Rapids	ĭ		ŏ	5	ō	7	ŏ	ō	ŏ	7	43
Wisconsin:		1									•
Kenosha	0	' <i>-</i>	0	4	1	2		0	0	21	7
Madison Milwaukee	1		0	38 6	6	51	ŏ	7	ŏ	53	105
Bacine	ō		ŏ	ŏ	1	5	Ŏ	i	Ŏ	19	14
Superior	0		Ó	1	0	Û	0	0	0	14	6
Minnester		l						1			
Dukith	0		0	22	2	2	0	0	0	42	20
Minneapolis	ž		1 ľ	42	4	41	Ŏ	1	i	47	87
St. Paul	0		0	178	0	22	0	2	0	68	62
Iowa:	2			0		3	0	-	0	0	90
Sioux City	ŏ			2		ĭ	ĭ		ŏ	ŏ	
Waterloo	Ō			3		2	1		0	0	
Missouri:				90		- 04			•	,	
St Ioseph			0	39	57	20 1	ŏ	ő	ŏ	1	70 25
St. Louis	16	1	ŏ	152	7	10	ŏ	7	Š	7	16
North Dakota:		_									
Fargo	0		0	0 0	0	0	0		0	0	9
Grand Fores	0			U	U U	1	U	l V	U U	5	
A berdeen	0		0	0	0	0	0	0	0	0	
Nebraska:										_	
Omaha	3		0	166	8	6	0	3	0	5	51
Kansas:	0		6	90	2	1	0	1	0	4	15
Wichita	1		ŏ	1	ō	ī	ž	ī	ŏ	1Õ	24
	_		-								
Delaware:		ì		10			•	,			95
Maryland:	1		U	10		-	U	1	° I	•	
Baltimore	3	2	1	13	12	78	0	13	1	33	188
Cumberland	0		0	13	2	0	0	0	0	0	11
Frederick	0		0	U	0	0	U		- 1	-	1
Washington	0		0	21	8	10	0	11	0	11	131
Virginia:	-		-		-					_	
Lynchburg	1		0	52	0	0	0	1	0	5	11
Richmond	1		0	Ð ∡	1	3	ŏ	7	Ň	21	61
Roanoke	ō		ŏ	3	ő	i	ŏ	4	ŏ	ī	18
West Virginia:	-										-
Charleston	0		0	0	0	2	0		0	1	8
Wheeling	1		0	5	Ň	3	ŏ	ŏ	ĭ	i	
North Carolina:	v		Ŭ		Ŭ	Ŭ	Ů	Ĩ	-	-	Ũ
Raleigh	0		0	4	0	4	0	1	0	0	13
Wington Solom	0		0 0	29	8	P P	Ň	2		2	7
South Carolina:			U	. 10	, v		v	~	•	-	•
Charleston	0		0	0	0	0 /	0	3	0	7	31
Columbia	0		0	0	1	0	0	1	0	<u>o</u>	8
Georgia:	U		0	6	2	0	0	0	2	•	a
Atlanta	2	7	1	27	0	0	0	2	6	44	63
Brunswick	ō		ō	Ö	Ō	Õ	Ó	0	Q	0	1
Savannah	0	8	0	0	2	1	0	2	0	3	34
Miami	1		0	6	2	0	0	1	ol	22	15
Tampa	ō	2	2	ŏ	ĩ	ŏ	ŏ	ī	ŏ	ō	34
Kantusha											
Ashland			n	1	6	5	6	0	1	6	
Lexington	ŏ		ŏ	ô	ŏ	ŏ	ŏ	ĭ	ô	ž	10
Louisville	2		Ó	19	5	16	0	2	0	3	67
Tennessee:	,		•	RA	5	,	,	19	R	AR	87
Nashville	ôl		i	7	ĭ	ő	ô	ĩ	ĭ	1	40

Chate and alter	Diph-	Diph- theria		Mea-	Pneu-	Scar- let	Small-	Tuber-	Ty- phoid	Whoop- ing	Deaths,
State and city	cases	Cases	Deaths	Ca.ses	deaths	fever cases	cases	deaths	fever cases	cough cases	causes
Alabama: Birmingham Mobile Montgomery	1 0 0		0	2 2 6	3 0	2 0 0	0	3 1	3 0 0	10 0 7	55 22
Arkansas: Fort Smith Little Rock Louisiana:	0		0	0 124	0	1 0	0	2	0 0	0	3
New Orleans Shreveport Oklahoma:	6 0	3	3 0	6 2	5 0	5 1	0	9 1	2 0	4	103 40
Tulsa Texas:	0		0	68	Ó	2	0	0	1	21	
Dallas Fort Worth Galveston Houston San Antonio	3 3 4 0		0 0 1 2	1 0 0 11	0 3 2 4 3	8 2 0 1 0	5 0 0 0	4 1 0 5 7	0 0 1 0	0 1 0 0 0	63 31 17 65 83
Montana: Billings Great Falls Helena Missoula	0 0 0 0	 1	0 0 0 1	0 1 0 26	0 1 0 0	0 0 0 0	0 0 0 0	0000	0 1 0 0	000000000000000000000000000000000000000	4 4 1 8
Idaho: Boi se	0		0	1	0	0	0	0	0	0	6
Colorado: Denver Pueblo	1 0	23	0	7 0	8 0	12 1	0 0	7 0	1 0	3 3	72 8
Albuquerque Utah:	1		0	12	1	0	0	1	1	0	9
Salt Lake City Nevada: Reno	0		0	17 0	1 0	3 0	0	0	0	30 0	
Washington: Seattle Spokane Tacoma	0 0 0		 1	1 0 2	2	16 3 2	0 0 0	0	0 0 0	9 0 0	
Oregon: Portland Salam	0 0		0 0	3 7	4 0	6 1	3 0	2 0	0	3 0	57
Camornia: Los Angeles Sacramento San Francisco	20 0 3	16 1	3 0 1	376 2 3	7 3 6	43 1 5	8 0 0	23 7 17	0 0 1	56 81 81	278 175

City reports for week ended May 27, 1933-Continued

· State and city	Meningococcus meningitis		Polio- mye- litis	State and city	Mening meni	Polio- mye- litis	
	Cases	Deaths	cases		Cases	Deaths	Cases
Maine: Portland	1	1	0	Minnesota: St. Paul Missouri:	0	1	0
New York: New York New Jersey:	3	4	2	St. Louis Nebraska: Omaha	1	0	0
Newark	1	0	0	Georgia: Atlanta	1	1	0
Indianapolis	3	2	0	Washington: Seattle	2	o	1
Chicago	18	4	0	Portland	0	0	1
Detroit Wisconsin:	1	0	0	Sacramento	1	0	0
Milwaukee	1	2	1	1		1	1

Lethargic encephalitis.—Cases: New York, 2; Philadelphia, 1; Detroit, 1. Pellagra.—Cases: Baltimore, 1; Washington, 1; Wilmington, 1; Charleston, S.C., 1; Montgomery, 1; New Orleans, 2; Los Angeles, 1; Sacramento, 1; San Francisco, 1. Typhus fever.—Cases: Charleston, S.C., 1; Savannah, 1; Mobile, 1; Houston, 1.

FOREIGN AND INSULAR

CANADA

Provinces—Communicable diseases—2 weeks ended May 20, 1933.— The Department of Pensions and National Health of Canada reports cases of certain communicable diseases for the 2 weeks ended May 20, 1933, as follows:

Disease	Prince Ed- ward Island	Nova Scotia	New Bruns- wick	Que- bec	On- tario	Mani- toba	Sas- katch- ewan	Alberta	British Colum- bia	Total
Cerebrospinal men-			2	1	1					
Chicken pox		13	27	329	420	52	54	11	134	1.040
Diphtheria		2	4	46	6	11	2	2	3	76
Erysipelas				7	12	3		6	2	30
Influenza		3		5	4				16	28
Measles	3	5	15	499	835	6	5		23	E91
Mumps		1			395	50	30		64	540
Paratyphoid fever					6					6
Pneumonia		10			19		8		5	42
Poliomyelitis			1	1	1			1		4
Scarlet fever		11	2	103	133	15	13	13	13	303
Smallpox					1		5		1	7
Trachoma									25	25
Tuberculosis	10	7	11	164	48	22	37	5	46	350
Typhoid fever			3	60	14	6	1	1	3	88
Undulant fever					2					2
Whooping cough				112	191	S 9	22	11	17	442

CUBA

Provinces—Communicable diseases—4 weeks ended April 29, 1933.— During the 4 weeks ended April 29, 1933, cases of certain communicable diseases were reported in the provinces of Cuba as follows:

Disease	Pinar del Rio	Habana	Matan- zas	Santa Clara	Cama- guey	Oriente	Total
Chicken por Diphtheria Malaria Measles Scarlet faver	1 	4 5 4 2	1 1 14 19	3 8 51 10	5 1 46 4	8 1 25 5	22 16 141 40
Tetanus, infantile Tuberculosis Typhoid fever	1 3 5	27 7	21 9	29 30	1 7 8	1 41 19	3 128 78

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DENMARK

Communicable diseases—January-March 1933.—During the months of January, February, and March 1933, cases of certain communicable diseases were reported in Denmark as follows:

		Cases			
Disease	January	February	March		
Cerebrospinal meningitis	3 63	7	4		
Diphtheria and croup	378	214 249	204 281		
German measles	11	25 721	30 760		
Gonoriea Influenza	43, 063	51, 273	25, 530		
Measles	1, 649	1, 427	1, 003		
Paratyphoid fever	4	10	17		
Puerperal fever	11	13	16		
Scarlet fever	264	161	240		
Tetanus	4		1		
Typhoid fever Undulant fever (Bact. abort. Bang)	42	39	32		
Whooping cough	1, 619	1,422	1, 236		

FRANCE

Vital statistics—Years 1931 and 1932.—During the years 1931 and 1932, births, deaths, marriages, and divorces, were reported in France as follows:

	1931	1932
Number of marriages	326, 538 21, 212 730, 249 28, 058 680, 710 55, 444	314, 878 21, 848 722, 246 27, 537 660, 882 55, 177

Note.-The population of France was estimated as 41,835,000 during 1931.

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

(NOTE.—A table giving current information of the world prevalence of quarantinable diseases appeared in the PUBLIC HEALTH REPORTS for May 26, 1933, pp. 586-596. A similar cumulative table will appear in the PUBLIC HEALTH REPORTS to be issued June 30, 1933, and thereafter, at least for the time being; in the issue published on the last Friday of each month.)

Cholera

Philippine Islands.—During the week ended June 3, 1933, cholera was reported in the Philippine Islands as follows: Province of Bohol, 40 cases, 20 deaths; Province of Cebu, 1 case.

During the 3 weeks ended May 20, 1933, 29 cases of cholera with 23 deaths were reported in the Island of Samar, Philippine Islands.

Smallpox

Bolivia.—During the month of April 1933, 18 cases of smallpox were reported at La Paz, Bolivia; 3 cases at Potosí; several cases in the Department of Potosí; and isolated cases in the Department of Chuquisaca, Bolivia.

Mexico.—During the latter part of May 1933, smallpox was reported in the vicinity of Camaron, Mexico. Camaron is on the Don Martin Dam (or lake), about 70 miles by highway southwest of Nuevo Laredo, Mexico.

Typhus Fever

Bolivia.—During April 1933, 167 cases of typhus fever were reported in La Paz, Bolivia; several cases in the Department of La Paz; isolated cases in Cochabamba; 14 cases in Oruro; and 9 cases in Potosí, Bolivia.

Mexico.—During the latter part of May 1933, cases of typhus fever were reported in the vicinity of Camaron, Mexico.